

## The Role of Foot and Mouth Disease Outbreak in 2012 on Egyptian Small Ruminants and Pigs

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**Abstract:** Foot and mouth disease virus (FMDV) outbreak attacked Egypt in 2012. The disease attacked ruminants and pigs. The current study investigated FMD infection in sheep, goats and pigs during this outbreak. Blood samples and oro-pharyngeal fluid were collected from 150 sheep, 80 goats and 65 pigs from Al Daqhaliya, Al Qalyoubiya, Al Sharqiya and Helwan governorates. 250µl of each sample was inoculated and propagated on bovine thyroid cell (BTY), positive cytopathic supernatant from these cells was subjected to antigen ELISA for further confirmation of FMDV. Meanwhile, serum samples were subjected to Virus Neutralizing Antibody Test. Samples were also subjected to RT-PCR for FMDV detection. Results showed that FMDV was detected in sera of 95 sheep, 50 goats and 50 pigs. ELISA indicated that highly significant result (53.75%) of the samples taken from goats were positive for ELISA and gave CPE in BTY cells, followed by sheep samples (highly significant 51.67%) while pigs samples gave 20% positive results. On the other hand, samples which gave negative CPE were passaged for the 2<sup>nd</sup> time on BTY cells where 10.67, 8.75 and 67.69% of the samples taken from sheep, goats and pigs were positive in CPE and ELISA, respectively. While 36.67, 37.5 and 23.08% of the samples taken from sheep, goats and pigs were negative for CPE and ELISA, respectively. VNT show that 63.3% (n=95), 62.5 (n=50) and 76.92(n= 50) percentage of serum samples taken from sheep, goats and pigs were positive for FMDV antibodies. RT-PCR confirmed the SAT2 serotype of FMDV.

**Key words:** Foot and Mouth Disease • Foot and Mouth Disease Virus • FMD • FMDV • Sheep • Goat • Pigs

### INTRODUCTION

Foot-and-Mouth Disease (FMD) is a highly contagious viral disease which affects all cloven-hoofed domestic animals including cattle, sheep, goats, pigs and buffalo [1]. The effects of this disease have been far-reaching, not only on animal health and rural economy, but also on the prospects of livestock industry and international trade. Sheep and goats together (small ruminants) comprise the majority of the world's FMD-susceptible animal population. Although FMD in small ruminants is usually milder compared to cattle or pigs, the subclinically infected sheep and goats have been incriminated in the transboundary spread of the disease on several occasions in the past [2]. The disease spreads by contact between infected and domestic animals, by

animal products (milk, meat and semen), by mechanical transfer on people, wild animals and birds, by vehicles and fomites and by the airborne route [3-6]. A review of aspects of airborne spread of FMD is given at the paper on pathogenesis and diagnosis of FMD by Alexandersen *et al.* [7].

FMD outbreak attacked Egypt in 2012 and affected all cloven-hoofed animals, including sheep, goats, cattle, buffalo and pigs. An estimated 6.3 million buffalo and cattle and 7.5 million sheep and goats were at risk in Egypt. The causative agent was identified as FMD-SAT2 virus [8-12].

This study is aiming to investigate FMD in sheep, goats and pigs during the 2012 outbreak and attempt to find a suitable, accurate and fast method for the disease diagnosis.

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## MATERIALS AND METHODS

The current work was carried out during a period of one year (February, 2012-May, 2013). Collection of samples was carried out from Sheep, goats in three different governorates of Egypt which are Al Daqhaliya, Al Qalyoubiya and Al Sharqiya. Animals kept in small holder farms where there is no regular system of vaccination. While other samples were taken from illegal pig farms (because pig farming was banned since avian flu epidemic) at Helwan governorate in Egypt. Pigs fed on garbage and there were no system of vaccination.

**Animals:** Diseased animals showed vesicles in and around the mouth, on feet, on the snout of pigs, teats and vulva. Lameness and inappetence are the main symptoms in sheep and goats where the disease seemed to be a lot less in severity. Clinical examination was adopted on 150 sheep, 80 goats and 65 pigs.

**Sample Collection and Processing:** Clotted blood was collected for serology to detect antibodies. The sera were separated, inactivated at 56°C for 30 min and stored at 20°C until further use. Heparinized blood was collected and was processed immediately without storage for virus isolation (VI) and for RT-PCR. Probang samples (oropharyngeal fluid-OPF) were collected from the upper oesophagus and pharynx with a small probang sampling cup. Samples were stored at -20°C.

**Cell Lines and Virus:** Bovine thyroid cell culture (BTY) was provided by Vaccines and Serum Institute, VACSERA, Egypt, while reference virus and serum was kindly supplied from Animal Vaccines and Sera Institute, Abasia, Egypt.

**Virus Isolation and Propagation:** Heparinized blood, nasal secretions and probang samples were examined for the presence of live virus by BTY cells. Cell culture inoculation was performed according to [13-14]. BTY cells were inoculated with 250µl of the sample and incubated for 30 min at 37°C. The prescriptions were then gently washed with 0.04 M phosphate buffer containing antibiotics then 5 ml of maintenance medium was added prior to incubation at 37°C. At 24, 48 and 72 h post-inoculation (PI), cells were examined for cytopathic effect (CPE). The presence of FMDV in cultures showing CPE was confirmed using an antigen ELISA [15]. BTY cell

culture supernatants from samples showing no sign of CPE after 72 h were pooled and re-passaged once and the absence of FMDV was confirmed by the antigen ELISA.

**Virus Neutralizing Antibody Test (VNT):** Virus neutralization tests were performed for the sera in flat-bottomed tissue culture grade micro titer plates (Nuncclon™, Denmark) as described previously [16]. Antibody titers were expressed as the reciprocal of the final dilution of serum in the serum/virus mixture which neutralized an estimated 100 TCID<sub>50</sub> of virus at the 50% end-point [17].

**RT-PCR for FMDV diagnosis:** Virus RNA was isolated by QIAGEN® purification kit (Qiagen Co., Valencia, CA, USA). RT-PCR was performed using QIAGEN® one-step RT-PCR kit (Qiagen Co., Valencia, CA, USA). The primers used were targeting VP1 gene following the methodology outlined by Bastos [18] 5' CCA CGT ACT ACT TYT CTG ACC TGG A3' [18] and 5' GAA GGG CCC AGG GTT GGA CTC 3' primers targeting the highly conserved 2A/2B junction [19]. The steps were performed according to manufacturer instructions.

## RESULTS AND DISCUSSION

**Clinical Signs:** FMD is very recognizable disease from its pathognomonic lesions in pigs while it occurs in sheep and goats with almost with no observed symptoms except for Lameness and in-appetence. FMDV was detected in sera of 95 sheep, 50 goats and 50 pigs (Table 1). The symptoms described in this study matches with that reported by Madhanmohan *et al.* [20] and Alexandersen *et al.* [7]. Table 2 shows the different symptoms observed in clinical examination.

Mortalities among animals were very high especially among young ages. Tiger's heart was most common feature in post mortem examination in young age (Fig 1).

**Isolation and Propagation on Tissue Culture:** FMD virus causes cell degeneration within 72 hrs. CPE sometimes did not show in the first passage but they were very prominent in the second passage or when the prescriptions were left for long period 4-6 days PI (Fig 2). Furthermore, in the majority of experiments reported from 1981 onwards, BTY tissue cultures (the most sensitive system) were used for virus assay [21].

Table 1: Results of clinical examination

	Sheep		Goats		pigs	
	Clinically positive	No apparent signs	Clinically positive	No apparent signs	Clinically positive	No apparent signs
Numbers	13	137	7	73	52	15
percentage	8.67%	91.3%	8.75%	91.25%	80%	23.08%
$\chi^2$ value						12.32**

\*\*P<0.01

Table 2: Clinical symptoms in different animals

Clinical signs	Sheep	Goats	Pigs
Fever	-	-	41
In-appetent	10	7	25
Lameness	9	3	39
Salivation	-	-	46
Ulcer or vesicles on muzzle or snout	3	2	39
Ulcer or vesicles on coronary band	9	3	41
Ulcer or vesicles on teat	-	-	25
$\chi^2$ value	7.92**		

\*\*P<0.01

Table 3: ELISA results summary

	Samples show no CPE in BTY cells											
	Samples gave CPE in 2 <sup>nd</sup> passage											
	Samples show CPE in BTY cells and positive ELISA			Samples positive ELISA			Samples negative ELISA			Samples gave no CPE in 2 <sup>nd</sup> passage and negative ELISA		
	Samples from Sheep	Samples from Goats	Samples from pigs	Samples from Sheep	Samples from Goats	Samples from pigs	Samples from Sheep	Samples from Goats	Samples from pigs	Samples from Sheep	Samples from Goats	Samples from pigs
Number	79	43	13	16	7	44	-	-	2	55	30	15
Percent	52.67%**	53.75%**	20%	10.67%	8.75%	67.69%**	-	-	3.08%	36.67%	37.5%	23.08
$\chi^2$ value	23.04**											

\*\*P<0.01



Fig. 1: Shows tiger's heart in post mortem examination

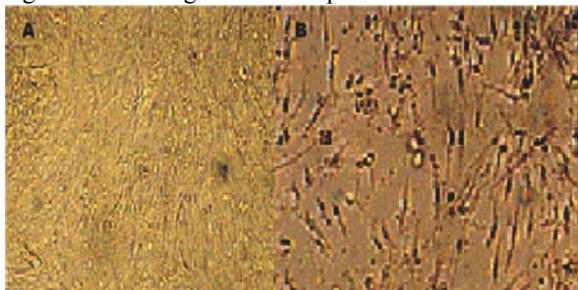


Fig. 2: Photo show normal BTY cell (A) and 72hrs PI.

**ELISA and VNT Tests:** Antigen ELISA results show that all the samples that gave CPE on tissue culture gave positive results of ELISA. Moreover, highly significant result (53.75%) of the samples taken from goats were positive for ELISA and gave CPE in BTY cells, followed by sheep samples (highly significant 51.67%) while pigs samples gave 20% positive results. On the other hand, samples which gave negative CPE were passaged for the 2<sup>nd</sup> time on BTY cells where 10.67, 8.75 and 67.69% of the samples taken from sheep, goats and pigs were positive CPE and ELISA, respectively. While 36.67, 37.5 and 23.08% of the samples taken from sheep, goats and pigs were negative for CPE and ELISA, respectively (Table 3). Similar results were obtained by Madhanmohan *et al.* [22].

VNT show that 63.3% (n=95), 62.5 (n=50) and 76.92 (n= 50) percentage of serum samples taken from sheep, goats and pigs were positive for FMDV antibodies.

Fig. 3 summarizes these results. The results of ELISA and VNT were identical which comes in agreement with the results obtained by Madhanmohan *et al.* [22].

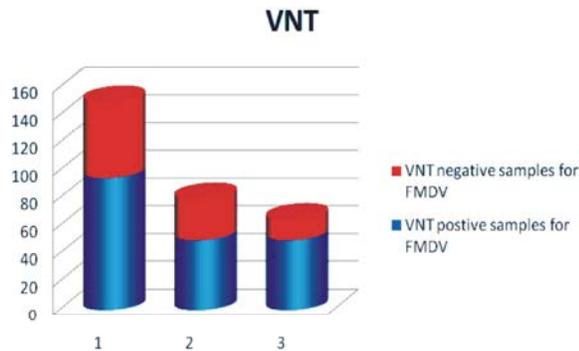


Fig. 3: Shows results of VNT.



Fig. 4: Photo shows agarose gel where a band at 518 bp was observed amplifying VP1 gene of FMD SAT-2 virus. Marker ladder (lane 1) is followed by negative control sample (lane2), control positive (lane8)

**Diagnosis of FMDV by RT-PCR:** Blood samples which their sera were positive by VNT and CPE and antigen ELISA test were subjected to RT-PCR test and positive band at 518 bp targeting the VP1 gene was observed (Fig 4). The results matches those reported by Golding *et al.*, [16]; Beck and Strohmaier [19].

In the last few years FMD infection in sheep and goats was not recognizable due to the phenomenon of virtually silent infection in these two species. In 2012 FMD outbreak attacked Egypt, noticeable increase of morbidity and mortality rates were justified by the new serotype SAT2 attack where there were no previously report of it in Egypt. This study recommends the screening of samples by ELISA or VNT and conformation by Rt-PCR.

El-Sayed *et al.*, [23] reported the presence of FMD O and A serotype which urge the use of multivalent vaccines in sheep, goats and pigs. The vaccination of these species is very important to control the disease in large ruminant animals because these animals are kept together in farm of small holders.

In conclusion, the study supported the findings that FMD SAT2 as the causative agent for 2012 outbreak in Egypt infecting sheep, goats and pigs. This study also confirms the significant role of these three species in the epidemiology of FMD in Egypt.

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